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Ultraviolet Resonance Raman Study of Lipid Mediated Peptide Folding
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The interactions between proteins and biological membranes play an important role in many aspects of biochemistry. Thus, the ability to monitor the structural dynamics of membrane proteins is of great interest. In general, hydrophobic peptides are disordered and tend to aggregate in aqueous environments. For example, the amyloid-β (Aβ) peptide, a major component of the insoluble plaques associated with Alzheimer's disease, is intrinsically disordered under physiological conditions. However, Aβ adopts α-helical structure in membrane mimicking environments. This is not surprising as the hydrophobic region derives from the transmembrane (TM) region of the amyloid precursor protein. More interesting is the fact that low concentrations of organic solvents or surfactants promote aggregation and formation of β-sheet structure. The ability to simultaneously monitor lipid association and study its effect on the secondary structure of amyloidogenic proteins would be of great interest. Recent studies have shown a significantly enhanced amide I mode in the deep-UV resonance Raman (dUVRR) spectra of transmembrane proteins is a marker for lipid association. Positively charged hydrophobic peptides, including the hydrophobic Aβ(25-40) fragment of Aβ, spontaneously insert into anionic lipid bilayers. The application of dUVRR spectroscopy to monitor lipid-association, insertion and folding of these peptides will be presented.

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Analysis of the Area Per Lipid in Protein-Membrane Systems
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Molecular dynamics (MD) simulation is a powerful research tool to investigate structural and dynamical properties of biological membranes and membrane proteins. The lipid structures of simple membrane systems in recent MD simulations are in good agreement with those obtained by experiments. However, for protein-membrane systems the complexity of protein-lipid interactions makes investigation of lipid structure more difficult. Although the area per lipid is one of the important structural properties of membranes, the area in protein-membrane systems cannot be calculated easily by conventional approaches like Voronoi tessellation method. Here, we developed a new method, based on a combination of the two-dimensional Voronoi tessellation and Monte Carlo integration methods. Monte Carlo integration enables us to estimate the cross-sectional area of arbitrary-shaped target molecules. We applied the method to all-atom MD trajectories of the sarcoplasmic reticulum Ca2+-pump and the SecY protein-conducting channel. The calculated lipid surface area was in agreement with experimental values and consistent with other structural parameters of lipid bilayers. We also observed a response of lipid bilayers to the conformational transition of SecY. We believe that our method is useful to analyze time courses of protein-lipid interactions in MD simulations of membrane proteins.

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Docking of PKC(Alpha)-C1 and PKC(Beta)-C2 Domains to POPC/POPS/POG Lipid Membranes
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Protein kinase C (PKC) isoenzymes are a large family of serine/threonine kinases that play important roles in cellular signaling. However, the detail process of their docking at cell membranes is still not well understood. In this study, eight independent atomistic molecular dynamics (MD) simulations were performed to systematically investigate the docking of PKC(alpha)-C1 and PKC(beta)-C2 domains to lipid bilayers composed of POPC, POPS, and POG (1-palmitoyl-2-oleoyl-sn-glycerol). POG concentration varied from 0 to 25% (i.e. 0%, 6.25%, 12.5%, and 25%), and the ratio of POPC/POPS was kept at 3/1. Our result shows that PKC(beta)-C2 domain adapts a parallel configuration when it docks to the lipid bilayer in the absence of POG; however, at the presence of POG, it adapts a perpendicular configuration. This difference in docking configurations is due to the increase of spacing between lipids headgroups at the presence of POG, which allows the perpendicular docking of PKC(beta)-C2 domain. Furthermore, PKC(beta)-C2 domain shows no significant conformational change during the docking in both cases. On the other hand, PKC(alpha)-C1 domain only docks to a lipid bilayer that contains POG. In the absence of POG, the domain stays in a parallel configuration on the surface of the bilayer. The docking of PKC(alpha)-C1 domain to lipid bilayers containing POG shows a high specificity of PKC(alpha)-C1 domain toward diacylglycerol.

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Organization and Lipid Interaction of the Model Amphipathic α-Helix Bundle Protein apoLp-III
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Amphipathic α-helix bundle domains are found in many proteins responsible for neutral lipid transport and storage. Important examples are found in apoE, the perilipins, and the representative exchangeable apolipoprotein apoLp-III. Here we characterized the organization and lipid interaction of apoLp-III in Langmuir monolayers modeling the phospholipid monolayer surrounding the neutral lipid particle, i.e. lipoprotein. Surface-sensitive X-ray techniques showed that apoLp-III is partially unfolded at the interface as the unfolded protein was best represented by two distinct regions. This surprising result does not result from the high degree of glycosylation of apoLp-III as the recombinant protein behaved in a similar fashion. This suggests that either apoLp-III is partially unfolded or that unfolded protein is associated with the protein monolayer. Injection of apoLp-III underneath a previously formed (phospho)-lipid monolayer results in a rapid increase of the surface pressure. We characterized this increase in pressure as a function of effective lipid molecular shape and lipid packing density. These results should shed important light on the interaction of amphipathic α-helix bundle domains with phospholipid monolayers. This work will be extended in the future to include additional apolipo-and lipid droplet proteins containing amphipathic α-helix bundle domains.

Membrane Dynamics & Bilayer Probes I

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Membrane Fluidity Profiles as Deduced by Saturation-Recovery EPR Measurements of Spin-Lattice Relaxation Times of Spin Labels: Multifrequency Approach
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New capabilities using saturation-recovery (SR) EPR at X-band (9.4 GHz) and W-band (94 GHz) to obtain profiles of the membrane fluidity have been demonstrated for dimyristoylphosphatidylcholine (DMPC) membranes with and without 50 mol% cholesterol. Phosphatidylcholine (n-PC) spin labels were used. Results were compared with profiles of the rotational diffusion coefficient, R_{perp}, obtained from simulation of EPR spectra using Freed's model. The spin-lattice relaxation rate (T_1^{-1}) obtained from SR EPR measurements of phospholipid spin labels in deoxygenated samples depends primarily on the rotational correlation time of the nitroxide moiety within the lipid bilayer. Thus, T_1^{-1} can be used as a convenient quantitative measure of membrane fluidity that reflects membrane dynamics at a certain depth in the membrane. The order parameter, which is often used as a measure of membrane fluidity, describes the amplitude of wobbling motion of alkyl chains relative to the membrane normal and does not explicitly contain time or velocity. Thus, the order parameter can be considered as ''nondynamic''. It is shown that T_1^{-1} and R_{perp} profiles reveal changes in membrane fluidity that depend on the motional properties of the lipid alkyl chain. We find that cholesterol has a rigidifying effect only to the depth occupied by the rigid steroid ring structure and a fluidizing effect at deeper locations. These effects cannot be differentiated by profiles of the order parameter. Results demonstrate that SR EPR at W-band has the potential to be a powerful tool for studying samples of small volume, ~30 nL, compared with a sample volume of ~3 μL typically required at X-band.

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Phase Boundaries in Phosphatidylcholine Membranes Saturated and Oversaturated with Cholesterol
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Conventional and saturation-recovery EPR along with differential scanning calorimetry (DSC) were used to determine the cholesterol/phosphatidylcholine (Chol/PC) mixing ratio at which cholesterol bilayer domains (CBDs) and cholesterol crystals (CR) start to form in dimyristoyl-PC (DMPC) and 1-palmitoyl-2-oleoy-PC (POPC) membranes. The Chol/PC mixing ratio was changed from